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Physiologically Based Pharmacokinetic Modeling in Risk Assessment: Case Study With Pyrethroids

Pankajini Mallick,* Gina Song,* Alina Y. Efremenko,* Salil N. Pendse,* Moire R. Creek,† Thomas G. Osimitz,‡ Ronald N. Hines,§,1 Paul Hinderliter,¶,2, Harvey J. Clewell,‖ Brian G. Lake,‖‖ Miyoung Yoon,* and Marjory Moreau*,3

*ScitoVation, LLC, Durham, North Carolina 27713; †Moire Creek Toxicology Consulting Services, Lincoln, California 95648; ‡Science Strategies, LLC, Charlottesville, Virginia 22902; §US EPA, Office of Research and Development, Center for Public Health and Environmental Assessment, Research Triangle Park, North Carolina 27709; ¶Syngenta, Greensboro, North Carolina 27409; ‖Ramboll, Research Triangle Park, North Carolina 27709; and ‖‖Faculty of Health and Medical Sciences, University of Surrey, Surrey GU2 7XH, UK

[1]Present address: Penhook, VA 24137.
[2]Present address: Pharmacometrics-Alexion Pharmaceuticals, Inc., Boston, MA 02210.
[3]To whom correspondence should be addressed at ScitoVation, 100 Capitola Drive Suite 106, Durham, NC 27713. E-mail: mmoreau@scitovation.com.

ABSTRACT

The assessment of potentially sensitive populations is an important application of risk assessment. To address the concern for age-related sensitivity to pyrethroid insecticides, life-stage physiologically based pharmacokinetic (PBPK) modeling supported by *in vitro* to *in vivo* extrapolation was conducted to predict age-dependent changes in target tissue exposure to 8 pyrethroids. The purpose of this age-dependent dosimetry was to calculate a Data-derived Extrapolation Factor (DDEF) to address age-related pharmacokinetic differences for pyrethroids in humans. We developed a generic human PBPK model for pyrethroids based on our previously published rat model that was developed with *in vivo* rat data. The results demonstrated that the age-related differences in internal exposure to pyrethroids in the brain are largely determined by the differences in metabolic capacity and in physiology for pyrethroids between children and adults. The most important conclusion from our research is that, given an identical external exposure, the internal (target tissue) concentration is equal or lower in children than in adults in response to the same level of exposure to a pyrethroid. Our results show that, based on the use of the life-stage PBPK models with 8 pyrethroids, DDEF values are essentially close to 1, resulting in a DDEF for age-related pharmacokinetic differences of 1. For risk assessment purposes, this indicates that no additional adjustment factor is necessary to account for age-related pharmacokinetic differences for these pyrethroids.

Key words: PBPK modeling; pyrethroids; IVIVE; risk assessment.

Risk assessment is defined as the characterization of the potential adverse effects in humans to exposures of environmental hazards (NRC, 1983). Classic risk assessment processes include identifying a point of departure (POD) from animal toxicity studies and calculating a human reference dose using uncertainty factors generally applied to reflect limitations of the data used and to address variability and uncertainty from differences between and within test animals and humans. For pesticides

regulated by the U.S. Environmental Protection Agency (U.S. EPA), additional default uncertainty factors such as the Food Quality Protection Act Safety Factor can be applied to protect sensitive populations from risk. The Food Quality Protection Act safety factor is set by statute at a default value of X10 (U.S. EPA, 2014), but it can be modified with Data-derived Extrapolation Factors (DDEFs) if additional and reliable information can be provided to address the uncertainties.

The increased use of pyrethroids over the years has introduced new concerns, particularly concern over children's health resulting from pyrethroid exposure. Prompted by the passage of the Food Quality Protection Act in 1996, the U.S. EPA is required to separately assess children's sensitivity to pesticides, including pyrethroids. In the Agency's 2010 review of the pyrethroid toxicology database, age-dependent differences in sensitivity were noted in rats treated with high doses of pyrethroids (Cantalamessa, 1993; Sheets, 2000; Sheets *et al.*, 1994). This observation was supported with physiologically based pharmacokinetic (PBPK) models for adult and developing rats (Godin *et al.*, 2010; Mirfazaelian *et al.*, 2006; Tornero-Velez *et al.*, 2010, 2012). They concluded, however, that "It is unknown whether such sensitivity would occur at lower doses more relevant for human health risk assessment" (U.S. EPA, 2010), as a human age-dependent PBPK model was not available at that time. Based on this review, the U.S. EPA concluded that the existing studies did not adequately characterize potential susceptibility of the young human (Scollon *et al.*, 2011; EPA-HQ-OPP-2011-0746-0011) and decided to re-evaluate the Food Quality Protection Act Safety Factor for human health risk assessments for pyrethroid pesticides. They solicited protocols to develop data to address and quantify potential age-related human sensitivity. In response, the Council for the Advancement of Pyrethroid Human Health Risk Assessment was formed in 2011 to conduct a research program aimed at addressing whether children are more sensitive than adults to the acute neurotoxic effects of pyrethroids. During the time of this evaluation, the U.S. EPA retained a Food Quality Protection Act Safety Factor of 3 (based on age-related pharmacokinetic differences) for children less than 6 years of age (EPA-HQ-OPP-2011-0746-0003).

In keeping with the principles outlined in the landmark document, Toxicity Testing in the 21st Century (NRC, 2007), alternative strategies to traditional *in vivo* animal testing, such as *in vitro* and *in silico* approaches, have been incorporated into study designs for evaluating pyrethroid toxicity and risk. These include the use of *in vitro* to *in vivo* extrapolation (IVIVE) coupled with PBPK modeling. PBPK modeling takes a variety of inputs, some chemical dependent, others chemical independent, and incorporates them into the estimation of target tissue dose. PBPK models have been used over the past 3 decades to predict the internal dose of a chemical in the target tissue. The IVIVE and *in silico*-based parameterization strategies can be applied to build a generic PBPK modeling tool for chemicals, especially for human children where *in vivo* data generation is not possible (Yoon and Clewell, 2016). Life-stage IVIVE-PBPK modeling provides an improved platform as it appropriately incorporates species and age-specific profiles when evaluating age-specific internal dosimetry to support pyrethroid risk assessment in early ages.

To characterize the basis for the greater sensitivity expressed in young rats and assess its relevance to humans, we developed a generic life-stage IVIVE-PBPK model for pyrethroids (Mallick *et al.*, 2020). The purpose of the early age dosimetry with an IVIVE-PBPK model was to calculate a data-derived Food Quality Protection Act safety factor for the entire class of pyrethroids and address age-related pharmacokinetic differences for pyrethroids in humans. In this study, 8 pyrethroids were used as case compounds; deltamethrin, *cis*-permethrin, *trans*-permethrin, bifenthrin, β-cyfluthrin, λ-cyhalothrin, cyphenothrin, and esfenvalerate.

MATERIALS AND METHODS

PBPK Model Description

The structure of the human life-stage PBPK model for pyrethroids is shown in Figure 1. The model includes plasma and 6 tissue compartments: gastrointestinal tract, liver, fat, brain, and slowly and rapidly perfused tissue. The current model can simulate pyrethroid kinetics through oral and inhalation in single or multiple daily exposure scenarios and incorporates age-dependent human physiology, as well as maturation profiles of pyrethroid metabolism mediated by carboxylesterases and cytochrome P450 enzymes in liver. Key assumptions of this human model's structure and parameters are based on those evaluated in the rat model (Song *et al.*, 2019). All information relative to human model structure and parameterization as well as sensitivity analysis can be found in Mallick *et al.* (2020).

Model Simulation

Simulations were run in the interactive modeling platform, Population Lifecourse Exposure-to-Health-Effects Model (Pendse *et al.*, 2020). Simulations were run until periodic steady state was reached and maximal concentration (C_{max}) values at steady state were used as they are considered to be best correlated with the neurotoxic effects of pyrethroids (Moser *et al.*, 2016; Scollon *et al.*, 2011).

This work has been reviewed by the U.S. EPA and the result of the risk assessment can be found online at: https://www.epa.gov/ingredients-used-pesticide-products/2019-evaluation-fqpa-safety-factor-pyrethroids (last accessed on may 26, 2020), or in the U.S. EPA docket dedicated to pyrethroids: https://www.regulations.gov/docket? D=EPA-HQ-OPP-2008-0331 (last accessed on May 26, 2020).

Calculation Steps to Derive an Age-related DDEF

Figure 2 depicts the steps used to estimate the DDEF through the use of both the rat and human life-stage PBPK models.

Extrapolating the POD from rat to human through PBPK modeling. PODs were selected from the U.S. EPA Benchmark Dose analysis of the Wolansky *et al.* (2006) data (DER No. D422817, U.S. EPA), based on individual dose-response curves for *in vivo* motor activity in adult rats. Many studies suggest motor activity as a sensitive endpoint for developmental neural toxicity effects of pyrethroids (Ahlbom *et al.*, 1994; Eriksson and Fredriksson, 1991; Eriksson and Nordberg, 1990). Based on these results, the benchmark dose lower confidence limit (BMDL1SD) (1 SD means that the dose was calculated at which the motor activity change is equal to 1 SD from the control value) was used as the POD, which was equal to 1.5, 44.4, 3.1, 0.7, 1.2, 0.6, and 6.0 mg/kg body weight (BW) for deltamethrin, permethrin (*cis*-/*trans*-permethrin), bifenthrin, esfenvalerate, cyfluthrin, cyhalothrin, and cyphenothrin, respectively. The external POD for permethrin was obtained from rats dosed with a 40:60 mixture of *cis*-:*trans*-permethrin (Wolansky *et al.*, 2006).

These PODs were used in the rat PBPK model from Song *et al.* (2019) to estimate the maximal concentration (C_{max}) at the target tissue (brain) in rat for each pyrethroid. The rat pyrethroids

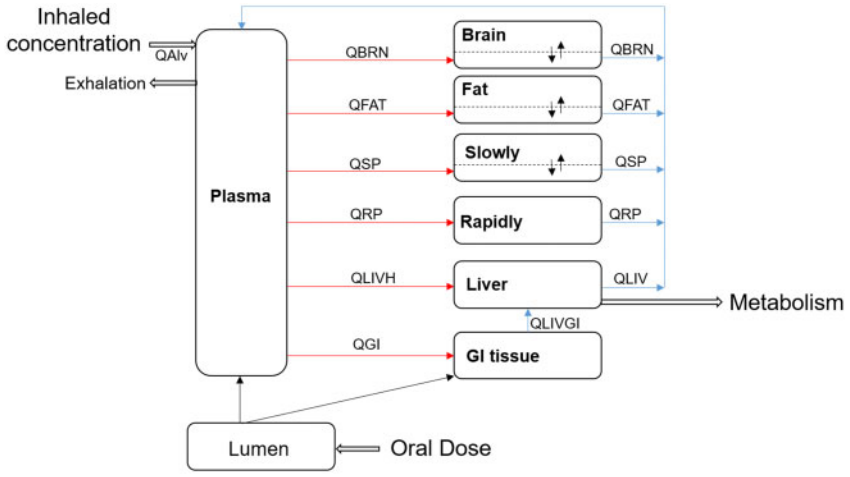

Figure 1. Structure of the life-stage pyrethroid PBPK model. QLIVGI, QLIVH, QRP, QSP, QFAT, QBRN refer to blood flow to each tissue compartment. QLIV is the sum of QLIVGI and QLIVH (the liver has a dual blood supply from arterial and venous blood). QALV refers to the alveolar ventilation rate. Brain, fat, and slowly perfused tissue compartments are described as diffusion-limited tissues, whereas all other tissue compartments are described as flow limited.

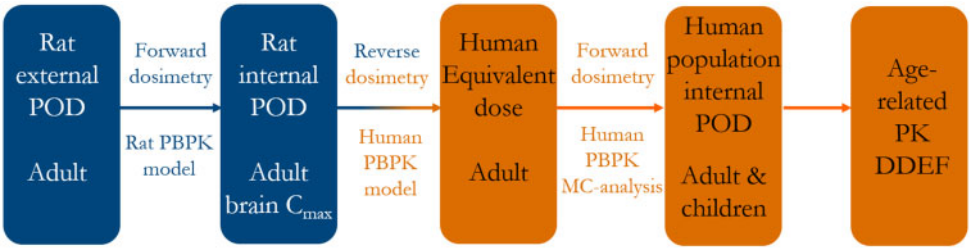

Figure 2. Calculation steps for development of Human Equivalent Dose from rat and age-related Data-derived Extrapolation Factor. Abbreviations: MC, Monte Carlo; PBPK, physiologically based pharmacokinetic; POD, point of departure.

model was run with a daily dose of 1.5, 44.4, 3.1, 0.7, 1.2, 0.6, and 6.0 mg/kg BW for deltamethrin, permethrin (*cis*-/*trans*-permethrin), bifenthrin, esfenvalerate, cyfluthrin, cyhalothrin, and cyphenothrin, respectively. The C_{max} estimated represents the rat internal POD. Applying reverse dosimetry with the human life-stage PBPK model, a dose referred as "Human Equivalent Dose (HED)" was estimated that resulted in similar C_{max} in human brain corresponding to the rat internal POD. Note that we used 1 single internal exposure estimate in the adult rat brain at the BMDL1SD for each pyrethroid to conduct reverse dosimetry for both early and adult ages in humans. Finally, forward dosimetry and Monte Carlo (MC) simulations were used to calculate age-related internal PODs from which the DDEFs were determined.

MC analysis. The human pyrethroids model was run with a daily oral dose (extrapolated from the rat PODs) with MC analysis in males of 5 different ages (0.5, 1, 5, 19, and 25 years old) to compare the internal exposure in the target tissue brain (C_{max}) across ages. MC simulations were performed with 1000 iterations, at which convergence was achieved. Further increase in the number of iterations to 5000 and 10 000 did not make a substantial difference in achieving convergence. MC analysis was performed to incorporate interindividual variation in the values of parameters across the population to predict the distribution of internal doses. Model parameters that varied for the MC analysis and their distributions are listed in Table 1 and were chosen based on the sensitivity analysis from Mallick *et al.* (2020). Information from published data shows that the coefficient of variation between children and adult for physiological parameters used in our MC analysis would not vary substantially (Price *et al.*, 2003), therefore the corresponding coefficient of variation for the parameters were kept similar across ages in our MC simulations.

DDEF calculation. DDEF was calculated using the age-specific internal dose metrics simulated by the life-stage PBPK model using MC analysis. The maximum concentration (C_{max}) in the brain was used as the most appropriate internal target tissue dose metric (Moser *et al.*, 2016; Scollon *et al.*, 2011). MC simulation was performed to generate the distributions of C_{max} at different ages, then DDEFs were calculated as: Juvenile C_{max}_50th percentile/Adult C_{max}_50th percentile. The risk assessment approach used in the manuscript is in accordance with standard U.S. EPA/Office of Pesticide Programs practice in determining a Food Quality Protection Act safety factor for early-life exposure to a chemical, which is based on a comparison of an average adult and an average juvenile, and differs in that way from the recommended calculation of an intraspecies DDEF for a sensitive population (U.S. EPA, 2018).

Different ages from 6 months old to adulthood were chosen based on published studies suggesting that children beginning as young as 6 months are exposed due to hand-to-mouth and/or object-to-mouth contacts (Freeman *et al.*, 2005; Reed *et al.*, 1999; Tulve *et al.*, 2002; Xue *et al.*, 2007; Zartarian *et al.*, 1997).

As the U.S. EPA recommends that the consistency of DDEF values should be evaluated over a range of doses surrounding the HED to increase the level of confidence, we used the HED as well as a 10-fold higher dose and a 10-fold lower dose than the HED to calculate the DDEF.

Table 1. Parameters Changed for the Monte Carlo (MC) Analysis

Parameters	Distribution[a]	Coefficient of Variation	Reference
Body weight	L	0.22	Price *et al.* (2003)
Hematocrit	L	0.06	Price *et al.* (2003)
Cardiac output (CARDOUTPC)	L	0.2	Thomas *et al.* (1996)
Fraction unbound in plasma	L	0.1	Sethi *et al.* (2014)
Brain fractional plasma flow (FRBRNC)	N	0.1	Price *et al.* (2003)
Brain partition coefficient (PBRN)	L	0.3	Clewell *et al.* (1999)
Brain permeability (PABC)	L	0.3	Assumption
Liver volume (VOLLIVERC)	N	0.16	Price *et al.* (2003)
Liver fractional plasma flow (FRLIVC)	N	0.16	Price *et al.* (2003)
Metabolic constant (VKM1C)	L	0.5	Thomas *et al.* (1996)
Empirical adjustment factor (KMF)	L	0.5	Assumption
Fat volume (VOLFATC)	N	0.41	Price *et al.* (2003)
Fraction absorbed to systemic circulation (SWH)	L	1.44	Calculated based on O'Driscoll (2002)

[a]Sample distributions obtained from Portier and Kaplan (1989) (N, normal L, lognormal). To avoid the selection of extreme outliers, distributions were symmetrically truncated. For the parameters with a normal distribution in MC, we applied a truncation above and below 2 SD from the mean. Parameters with a log-normal distribution are defined using the mean and SD of the log-transformed distribution. The truncation was applied to the transformed distribution in the same way as to the normal distribution.

Table 2. Calculated Rat and Human Point of Departure (POD) for the 8 Pyrethroids

Exposure	Deltamethrin	Permethrin	Bifenthrin	Esfenvalerate	Cyfluthrin	Cyhalothrin	Cyphenothrin	Notes
External POD in rats[a] (mg/kg/day)	1.5	44.4	3.1	0.7	1.2	0.6	6.0[c]	U.S. Environmental Protection Agency (U.S. EPA) DER (D422817) Wolansky *et al.* (2006)
Brain C_{max} (ng/g)[b]	28.3	1370 (*cis*-permethrin) 280 (*trans*-permethrin)	219.8	7.3	30.6	17.4	67.6	PND90 simulated
Human Equivalent Dose (mg/kg/day)	0.9	14.1 (*cis*-permethrin) 8.7 (*trans*-permethrin)	0.9	0.1	0.8	0.4	2.1	Adult simulated

Estimated adult brain tissue internal dose at the reported benchmark dose lower confidence limit (BMDL) as POD in adult rats from U.S. EPA based on the benchmark dose analysis of the Wolansky *et al.* (2006) data.
[a]External PODs in rats correspond to BMDL values which correspond to a Benchmark Response of 1 SD change from the control mean.
[b]Brain C_{max} was calculated separately assuming that dosing was with a single permethrin isomer (*cis*- or *trans*-permethrin) at the rat POD (44.4 mg/kg/day).
[c]No observed adverse effect level for neurological effects as external POD in rats for Cyphenothrin from WIL Laboratories Research (Ashland, Ohio) Functional Observational Battery study used by the Environmental Protection Agency in their risk assessment (Weiner *et al.*, 2009).

RESULTS

POD Estimation

The rat external PODs of 1.49, 3.13, 0.65, 6.0, 0.64, 44.4, and 1.17 mg/kg BW for deltamethrin, bifenthrin, esfenvalerate, cyphenothrin, cyhalothrin, permethrin (*cis*- and *trans*-permethrin), and cyfluthrin, respectively were translated into brain C_{max} using the rat PBPK model from Song *et al.* (2019). The estimated internal exposure PODs (brain C_{max}) were 28.3, 219.8, 7.3, 67.6, 17.4, 1371.8, 279.9, and 30.6 ng/g for deltamethrin, bifenthrin, esfenvalerate, cyphenothrin, cyhalothrin, *cis*-permethrin, *trans*-permethrin, and cyfluthrin, respectively (Table 2). Reverse dosimetry was then conducted using the human life-stage PBPK model (Mallick *et al.*, 2020) to determine the HED in adult (25 years old) yielding the brain C_{max} in rat. The daily oral doses (HEDs) used to run the model (until steady state at 120 days) and calculate the DDEF were 0.9, 0.9, 0.1, 2.1, 0.4, 14.1, 8.7, and 0.8 mg/kg/day for deltamethrin, bifenthrin, esfenvalerate, cyphenothrin, cyhalothrin, *cis*-permethrin, *trans*-permethrin, and cyfluthrin, respectively (Table 2).

MC Simulations

Figure 3 shows the distribution of brain C_{max} for deltamethrin, *cis*-permethrin, *trans*-permethrin, esfenvalerate, bifenthrin, cyphenothrin, cyfluthrin, and cyhalothrin after oral doses (0.9, 14.1, 8.7, 0.1, 0.9, 2.1, 0.8, and 0.4 mg/kg/day, respectively) at steady state for different ages (0.5, 1, 5, 19, and 25 years old) in 1000 male humans. Steady state is reached at 120 days so the distribution of the last C_{max} values for brain corresponding to 120 days was estimated. Because the diffusion (uptake) of pyrethroids across the blood-brain barrier is slower than plasma flow rate in the brain, the equilibrium between the plasma and the brain is not instantaneous. This does not reflect accumulation of the compound in brain but a delay in reaching equilibrium between plasma and brain. Thus, to predict absolute C_{max} after exposure to a pyrethroid through any route, the model needs to be run to reach steady state, in this case 120 days (each with oral dosing 1x/day), at which point equilibrium between plasma and brain is achieved. This does not alter the fact that acute neurotoxicity from pyrethroids is a consequence of brain C_{max}, not the total exposure to a pyrethroid

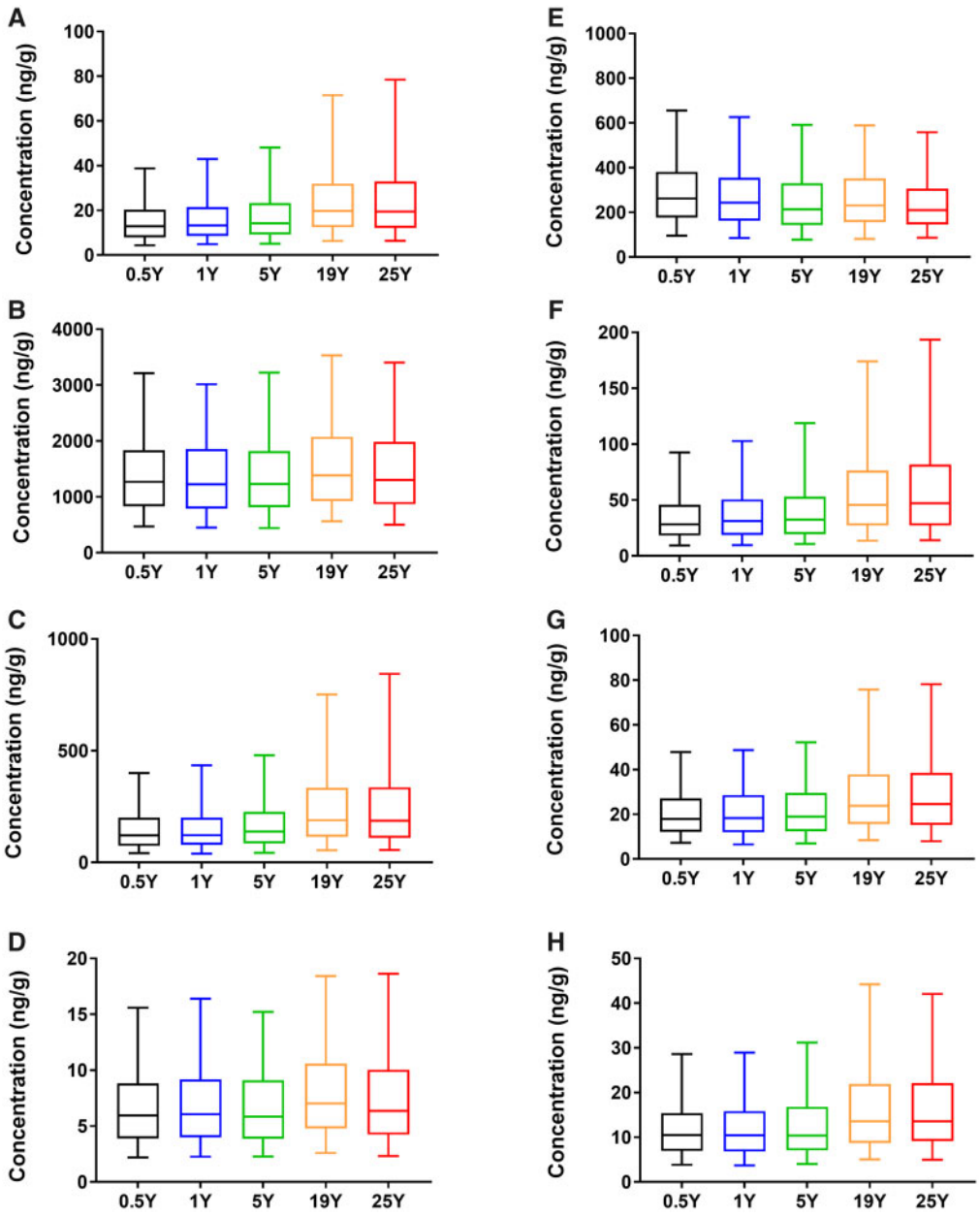

Figure 3. Brain C_{max} after a daily deltamethrin (A), *cis*-permethrin (B), *trans*-permethrin (C), esfenvalerate (D), bifenthrin (E), cyphenothrin (F), cyfluthrin (G), and cyhalothrin (H) oral dose at 0.9, 14.1, 8.7, 0.1, 0.9, 2.1, 0.8, and 0.4 mg/kg/day, respectively in male humans of 5 different ages during 120 days. One thousand individuals for each group were simulated for 120 days. Horizontal line bisecting large rectangle, median (50th percentile); large rectangle, lower and upper quartiles; whiskers, 5th and 95th percentiles.

over time. These results show that the internal exposure (C_{max}) at the target tissue (brain) for humans between 6 months and 25 years of age after oral exposure to the 8 pyrethroids would be lower at ages 0.5, 1, and 5 years than at 19 and 25 years. In addition, there are no significant sex differences in internal exposures (plasma and brain) between males and females (results not shown), consistent with a lack of sex differences in metabolism.

DDEF Calculation

Tables 3 and 4 show that for the 8 pyrethroids, the ratios between the 2 median C_{max} values are close to 1, resulting in a DDEF for age-related pharmacokinetic difference close to 1.

DISCUSSION

Pyrethroids are a group of insecticides that includes the natural pyrethrins and more than 30 synthetic compounds with a similar basic structure and activity. The parent pyrethroid is considered to be the toxicologically active compound and is rapidly absorbed, distributed, and cleared from the body. Although pyrethroids have relatively low mammalian toxicity, the relevant endpoint of concern for human risk assessment is a potential to cause acute neurotoxic effects (Chrustek *et al.*, 2018; Scollon *et al.*, 2011).

Interspecies differences can represent a major source of uncertainty in toxicology (Dohnal *et al.*, 2014) with differences in metabolism representing only 1 of several aspects which can

Table 3. Data-derived Extrapolation Factors at 3 Different Ages in Human Males for Deltamethrin, *cis*-Permethrin, *trans*-Permethrin, and Esfenvalerate in Brain and Plasma

		Deltamethrin		*cis*-Permethrin		*trans*-Permethrin		Esfenvalerate	
Dose	Age	Plasma C_{max}	Brain C_{max}	Plasma C_{max}	Brain C_{max}	Plasma C_{max}	Brain C_{max}	Plasma C_{max}	Brain C_{max}
HED	5 vs 25	0.82	0.73	1.08	0.94	0.83	0.73	1.13	0.92
+10-fold HED		0.82	0.76	1.17	0.96	0.78	0.70	1.14	0.92
−10-fold HED		0.90	0.78	1.13	0.97	0.85	0.72	1.07	0.86
HED	1 vs 25	0.86	0.68	1.13	0.94	0.82	0.65	1.23	0.95
+10-fold HED		0.86	0.71	1.17	0.94	0.79	0.67	1.19	0.90
−10-fold HED		0.94	0.76	1.20	0.96	0.85	0.68	1.09	0.86
HED	0.5 vs 25	0.81	0.66	1.21	0.97	0.82	0.65	1.25	0.93
+10-fold HED		0.82	0.65	1.22	1.00	0.80	0.65	1.23	0.90
−10-fold HED		0.92	0.69	1.18	0.96	0.87	0.69	1.14	0.86

The main value in the table is the results of the equation: Juvenile $C_{max\_50th\ percentile}$/Adult $C_{max\_50th\ percentile}$.
Human Equivalent Dose (HED) 0.85, 14.1, 8.73, and 0.1136 mg/kg/day for deltamethrin, *cis*-permethrin, *trans*-permethrin, and esfenvalerate, respectively; +10-fold HED indicates the use of a 10-fold higher dose than the HED; −10-fold HED indicates the use of a 10-fold lower dose than the HED.

Table 4. Data-derived Extrapolation Factors at 3 Different Ages in Human Males for Bifenthrin, Cyphenothrin, Cyfluthrin, and Cyhalothrin in Brain and Plasma

		Bifenthrin		Cyphenothrin		Cyfluthrin		Cyhalothrin	
Dose	Age	Plasma C_{max}	Brain C_{max}	Plasma C_{max}	Brain C_{max}	Plasma C_{max}	Brain C_{max}	Plasma C_{max}	Brain C_{max}
HED	5 vs 25	1.04	1.02	0.80	0.69	0.91	0.77	0.92	0.76
+10-fold HED		1.03	0.95	0.80	0.71	0.96	0.80	0.99	0.77
−10-fold HED		1.07	1.01	0.76	0.65	0.93	0.80	0.92	0.79
POD	1 vs 25	1.11	1.16	0.80	0.66	0.98	0.75	0.97	0.77
+10-fold HED		1.12	1.13	0.84	0.68	1.01	0.78	1.04	0.81
−10-fold HED		1.09	1.13	0.79	0.67	1.03	0.77	0.95	0.74
HED	0.5 vs 25	1.12	1.24	0.77	0.60	0.98	0.73	0.99	0.77
+10-fold HED		1.11	1.15	0.82	0.67	1.04	0.77	1.03	0.77
−10-fold HED		1.14	1.20	0.78	0.62	1.03	0.77	0.95	0.74

The main value in the table is the results of the equation: Juvenile $C_{max\_50th\ percentile}$/Adult $C_{max\_50th\ percentile}$.
Human Equivalent Dose (HED) 0.89, 2.13, 0.7706, and 0.44 mg/kg/day for bifenthrin, cyphenothrin, cyfluthrin, and cyhalothrin, respectively; +10-fold HED indicates the use of a 10-fold higher dose than the HED; −10-fold HED indicates the use of a 10-fold lower dose than the HED.
Abbreviation: POD, point of departure.

explain interspecies differences. Humans also respond differently to chemical exposures based on several factors that can be exogenous and/or intrinsic. Exogenous factors relate to exposure conditions such as chemical concentration/external dose, media, pathway, or dose duration. Physiological, anatomical, and biochemical parameters are intrinsic factors that may also be the basis for differential susceptibility among the population and at different life stages. Thus, life stage is a key consideration in susceptibility. A life-stage PBPK model for pyrethroids was developed to provide a scientific basis for assessing juvenile sensitivity (Mallick *et al.*, 2020). The primary goal of the model was to calculate a DDEF and determine whether the current Food Quality Protection Act Safety Factor (3x) for age-related pharmacokinetic differences should be retained. The DDEF was calculated using the age-specific maximum concentration (C_{max}) in the brain or in plasma. As explained in detail by Mallick *et al.* (2020), the model parameterization was achieved by adjusting the PBPK model domains on; (1) species-specific physiology, such as differences in organ size, perfusion, etc., (2) species-specific protein binding of pyrethroids, (3) kinetic parameters, such as V_{max} and K_M for the primary route of elimination, and (4) age-specific gene expression of the key metabolic enzymes. Species-specific differences in PODs were observed for all 8 pyrethroids (Table 2). With an identical external exposure, the internal (target tissue) concentration is lower in children than in adults resulting in lower extrapolated HEDs than observed rat PODs. Metabolism plays an important role in the detoxification and elimination of pyrethroids in both rats and humans, but species differences exist in the enzymes involved in pyrethroid metabolism (hepatic cytochrome P450 and carboxylesterases enzymes) as well as in the ontogeny of these enzymes (Boberg *et al.*, 2017; Crow *et al.*, 2007; Godin *et al.*, 2006, 2007; Hedges *et al.*, 2019a,b; Hideo, 2012; Hines, 2007; Hines *et al.*, 2016; Saghir *et al.*, 2012; Scollon *et al.*, 2009). At high doses, the species-specific immaturity of metabolizing enzymes in juvenile animals can result in the saturation of available metabolic capability, thus leading to higher pyrethroid concentrations in target tissues in juvenile rats compared with adults (Anand *et al.*, 2006; Kim *et al.*, 2010). Age-dependent differences in plasma levels of deltamethrin diminished as dose decreased, however, Mortuza *et al.* (2019) showed that age-dependent differences in brain levels persisted. Plasma carboxylesterases enzymes play an important role in pyrethroid metabolism in rats, but they are not present in humans (Crow *et al.*, 2007). In our study, when compared with rats, the total hepatic intrinsic clearance values (CL_{int}) for most of the pyrethroids were lower

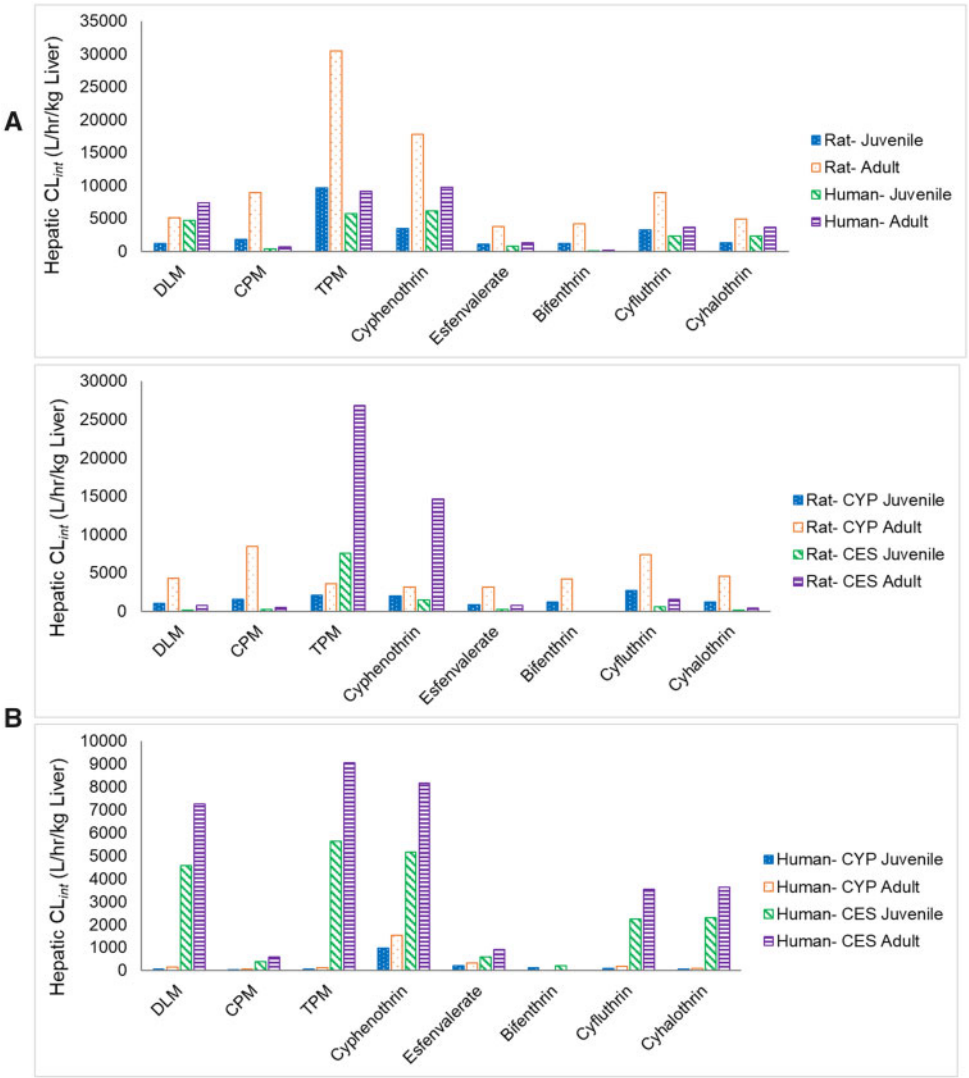

Figure 4. Species and age differences in the hepatic metabolism of the 8 pyrethroids. A, Comparison of total hepatic CL_{int} data for juvenile (PND15) and adult (PND90) rats and children (1Y) and adult (25Y) humans. B, Comparison of contribution of cytochrome P450 and carboxylesterases enzymes to hepatic pyrethroid metabolism in juvenile and adult rats and humans. The total intrinsic clearance of the 8 pyrethroids (deltamethrin, *cis*-permethrin, *trans*-permethrin, esfenvalerate, bifenthrin, cyphenothrin, cyhalothrin, and cyfluthrin) were assessed in rat and human liver microsomes and cytosol *in vitro* and scaled to *in vivo* CL_{int}. All the explanations and calculations can be find in Song *et al.* (2019) and Mallick *et al.* (2020).

in humans, except for deltamethrin (Figure 4A). Although cytochrome P450 enzymes mediated CL_{int} appeared to be the dominant pathway for deltamethrin, *cis*-permethrin, esfenvalerate, bifenthrin, cyfluthrin, and cyhalothrin, but not *trans*-permethrin and cyphenothrin, clearance in rats; in humans, carboxylesterases enzymes contributed significantly to the total hepatic CL_{int} for all pyrethroids except for bifenthrin (Figure 4B). The observed species-specific differences in metabolism of these pyrethroids results in pharmacokinetic differences between humans and rats, thus leading to difference in PODs and HEDs.

When looking at the differences in rat PODs and C_{max} values for each pyrethroids, it was interesting to look at their potency. Wolansky *et al.* (2006) published the ED30 for several pyrethroids. The ED30 is defined as the dose (mg/kg) required to induce a 30% decrease in total motor activity as compared with the corresponding vehicle-treated control group. Based on the observed ED30, the pyrethroids are ranking as follows: Esfenvalerate>cyhalothrin cyfluthrin>deltamethrin>bifentrhin>permethrin (*cis*- and *trans*-permethrin) (cyphenothrin was not part of that study). It seems that in our study, bifenthrin had one of the highest predicted brain C_{max} but is among the least potent pyrethroid based on the ED30; esfenvalerate had low predicted brain C_{max} but is the most potent pyrethroid based on the ED30. Cao *et al.* (2011) investigated *in vitro* the potency and efficacy of several pyrethroids to evoke sodium (Na^+) influx in neurons. The relative potency was calculated as the ratio of the ED30 for deltamethrin over the ED30 for each chemical. The rank order of potency was esfenvalerate>cyhalothrin>cyfluthrin>deltamethrin>bifenthrin, which agreed with the assumption that esfenvalerate is more potent than cyhalothrin and bifenthrin.

Intraspecies and interspecies extrapolation factors are applied for pharmacokinetics and pharmacodynamics. This manuscript outlines the application of a PBPK model in risk assessment that assesses potential age-related pharmacokinetic differences in humans and between species. This is especially crucial as it is well established that species differences exist in the enzymes involved in pyrethroid metabolism (Crow *et al.*, 2007; Godin *et al.* 2006, 2007; Hedges *et al.*, 2019b; Hideo,

2012; Scollon *et al.*, 2009) and in the ontogeny of the enzymes that play a major role in pyrethroid metabolism (Boberg *et al.*, 2017; Hines, 2007; Hines *et al.*, 2016; Saghir *et al.*, 2012). Intraspecies variation in pharmacokinetic is defined as differences in tissue concentration attained from the same human external exposure (eg, HED in this case) that results in different sensitivity among humans. The difference in internal tissue concentrations may be the result of altered distribution and elimination. Age-dependence of plasma protein binding of pyrethroids may play an important role in the distribution as Amaraneni *et al.* (2016) showed that the brain uptake of deltamethrin under normal physiological conditions appears to be a passive, nonsaturable process, limited by the high protein binding of the pyrethroid. However, as explained in Mallick *et al.* (2020), based on the study by Sethi *et al.* (2016), the fraction unbound in plasma was significantly higher (2–2.5×) in the birth to 1 week and > 1- to 4-week age groups, but reached and remained at adult levels in groups of infants and children older than 4 weeks. Because the youngest age group included in the PBPK model is 6-month-old infants, the age-dependent effects of plasma protein binding are not relevant to our simulations. Maximum concentration (C_{max}) and area under the curve are suitable measures for tissue concentrations. Acute toxicity of pyrethroids is highly correlated with the brain C_{max} values (Moser *et al.*, 2016; Scollon *et al.*, 2011) and not the total exposure to a pyrethroid over time, therefore, DDEFs were estimated as the ratio of C_{max} from the sensitive population to that for the adult population. Because the diffusion (uptake) of pyrethroids across the blood-brain barrier is slower than plasma flow rate in the brain, the equilibrium between the plasma and the brain is not instantaneous. This does not reflect accumulation of the compound in brain but a delay in reaching equilibrium between plasma and brain. Thus, to predict absolute C_{max} after exposure to a pyrethroid, the model was run for 120 days to reach steady state, at which point equilibrium between plasma and brain was achieved. Despite the limitations of the model, the way the FQPA safety factor is calculated do not account for difference in scenario of exposure. We use the ratio of (the median of the most sensitive age group)/(the median of the adult group) using the same POD for neurotoxicity effect in rats. The DDEFs were estimated for those ages deemed sensitive which includes ages from 6 months to 5 years old. Generally, once applied residentially, pyrethroids bind readily to the particulate matter in house dust and therefore particles resuspended by human activity then act as the primary vector. Exposure of young children, for whom indirect ingestion of residues from object- and hand-to-mouth activities is common, is subject to the highest levels of pyrethroids. Published studies have suggested that young children (2–5 years) exhibit higher hand-to-mouth and/or object-to-mouth contacts than older children and adults (Freeman *et al.*, 2005; Reed *et al.*, 1999; Tulve *et al.*, 2002; Xue *et al.*, 2007; Zartarian *et al.*, 1997). Such exposures to children below 6 months of age are negligible because they are less mobile and the levels of pyrethroids in food and drinking water are generally low (U.S. EPA, 2019). Furthermore, monitoring studies have shown that low (0.1 ng/g or less) or nondetectable levels of pyrethroids are found in human milk (Lehmann *et al.*, 2018). The modeling results showed that the internal exposure (C_{max}) at the target tissue (brain) in children after oral exposure to the 8 pyrethroids would be lower than in adult. This is explained by the fact that the maturation of enzyme expression occurs really early (before 6 months of age) for most of the enzyme implicated in the metabolism of pyrethroids. The total *in vivo* hepatic clearance of each pyrethroid in early ages would then be higher than that in adults (Mallick *et al.*, 2020). The most likely reason is the well-known observation that relative liver mass (liver mass/body mass) is higher in young children than it is an adult (Murry *et al.*, 1995). Given the high efficiency and rapid maturation of carboxylesterases, clearance of the pyrethroids is very efficient, leading to a blood flow-limited metabolism, in both the young and the adult. The lower internal (target tissue) concentration in children in response to the same level of exposure to a pyrethroid is due to both chemical-dependent factors such as metabolism and chemical-independent (physiologic) factors in children which include, higher liver weight as a fraction of BW and at a lesser degree, higher liver blood flow as a fraction of total blood flow (cardiac output) (Mallick *et al.*, 2020).

It is important to note that typically, risk assessments proceed from animal points of departure determined in the most sensitive species, strain, sex, and age. The dose-response data demonstrating neurotoxicity used to derive a POD in rats and subsequently a HED was from male adult rats (Wolansky *et al.*, 2006). Although data suggest that the developing rat is more sensitive to high dose effects (such as lethality) than adults, this is most likely due to less well-developed metabolizing enzymes in juveniles versus the adults. Considering that, the U.S. EPA guideline states that the basis for comparison of human variability is at the level of the internal dose metric (which drive the toxic response) rather than the external dose (U.S. EPA, 2014). Thus, the use of data from the adult rat as POD is appropriate. Metabolism and kinetic properties can vary across doses, particularly in the higher dose ranges; thus, we calculated multiple DDEF value estimates 10-fold above and below the POD to avoid potential uncertainty that may be introduced by nonlinearity in kinetic properties and to demonstrate the stability of the DDEF value. For all the pyrethroids, the ratios between the brain median C_{max} values from different ages are close to 1, resulting in a DDEF for age-related pharmacokinetic difference close to 1 for pyrethroids in brain. These results indicate that there is no additional adjustment factor required for age-related pharmacokinetic differences for these compounds. Read across principles applied to these findings indicate that no additional safety factor is required for age-related pharmacokinetic differences for the entire class of pyrethroid insecticides.

The life-stage PBPK model described in this work is a powerful platform that can be used to study target tissue levels of pyrethroids following exposure of juvenile and adult humans. Based on our application of the life-stage PBPK model developed and presented in Mallick *et al.* (2020), we have shown that the DDEF for age-related pharmacokinetic differences for pyrethroid exposure in humans can be reduced from X3 to X1.

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